



PATENT

Docket No. 265.00260101

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s): Watowich et al.) Group Art Unit: 1632
)
Serial No.: 09/981,286) Examiner: Unknown
Confirmation No.: 4993)
)
Filed: October 15, 2001)
For: DRUG DISCOVERY METHODS

**PRELIMINARY AMENDMENT,
COMMUNICATION REGARDING ENTRY OF SEQUENCE LISTING,
AND PROPOSED DRAWING CORRECTIONS**

Assistant Commissioner for Patents
ATTN: Missing Parts
Washington, D.C. 20231

Dear Sir:

Prior to taking up the above-identified application for examination, please amend the application as follows:

In the Specification

Please replace the paragraph beginning at page 20, line 20, with the following rewritten paragraph. Per 37 C.F.R. §1.121, this paragraph is also shown in Appendix A with notations to indicate the changes made.

The tat-CCD construct was produced by PCR using CCD in the pET30 vector and the primers N-TATCCD
(5'ATGTACGGTCGTAAAAACGTCGTCAGCGTCGTCGTGTCATGAAATTGGAATCTG
ACA3' SEQ ID NO:35) and CBAM-VEE
(5'GAATTCGGATCCTCATTACCATTGCTCGCAGTTCTCCGGAGT3' SEQ ID NO:36).
The PCR product was phenol-chloroform extracted and was ligated into the pETBLUE vector.
It was then transformed into NovaBlue Singles (Novagen) and plated on LB-Bluogal-IPTG-

**Preliminary Amendment, Communication Requesting
Entry of Sequence Listing, and Proposed Drawing Corrections**

Page 2

Applicant(s): Watowich et al.

Serial No. 09/981,286

Confirmation No. 4993

Filed: October 15, 2001

For: DRUG DISCOVERY METHODS

carbenicillin-tetracycline plates. White colonies were selected for amplification, plasmid purification, and sequencing. The tat-CCD cDNA sequence was determined and is depicted in Figure 3.

Please replace the paragraph beginning at page 26, line 28, with the following rewritten paragraph. Per 37 C.F.R. §1.121, this paragraph is also shown in Appendix A with notations to indicate the changes made.

Additional approaches to constructing the insert containing the library are also being used. One of these approaches involves annealing a negative strand of LIB (termed LIB r/c) to LIB itself. The LIB r/c sequence was (5'TCGAGGGAACCACC(MNN)mACCACCGGAG (SEQ ID NO:25)), where M= C, A. When LIB and LIB r/c were annealed, cohesive ends for BamHI and XhoI are formed. Another approach is to use Sequenase V 2.0 (USB, Cleveland, Ohio) to synthesize the negative LIB strand. The oligos for this are LIBSEQBAM (5'GCACGGATCCTCCGGTGGT(NNK)mGGTGGTTCCCTCGAGATCG (SEQ ID NO:26)) and SEQBAM Rev (5'CGATCTCGAGGGAACCATC (SEQ ID NO:27)). This sequenase product is then digested with BamHI (Promega, Madison, WI), and XhoI for insertion into the tat-CCD:BAM expression vectors.

SEQUENCE LISTING

In accordance with 37 C.F.R. §1.821 et seq., a computer readable form (CRF) and written Sequence Listing for the above-captioned application are submitted herewith. Applicants request entry of same into the specification.

In accordance with 37 C.F.R. §1.821 et seq., it is respectfully submitted that the written Sequence Listing and the Computer readable form of the Sequence Listing are identical. It is further submitted that Sequence Listing does not contain new matter.

**Preliminary Amendment, Communication Requesting
Entry of Sequence Listing, and Proposed Drawing Corrections**

Page 3

Applicant(s): Watowich et al.

Serial No. 09/981,286

Confirmation No. 4993

Filed: October 15, 2001

For: DRUG DISCOVERY METHODS

CORRECTION OF DRAWINGS

Applicants submit herewith proposed corrected drawings to replace originally filed sheets 3 and 4, which contain Figures 2B and 2C. The proposed corrections identify the sequences contained therein with the assigned SEQ ID NO. Additionally, at the bottom of Figure 2B, on the left hand side, text has inadvertently been omitted. Support for these corrections is found in SEQ ID NOS: 31, 32 and 12 of Figures 1 and 2A as originally filed. These changes are shown in red on the proposed corrected drawings submitted herewith. Approval of the proposed corrected drawings is respectfully requested.

**Preliminary Amendment, Communication Requesting
Entry of Sequence Listing, and Proposed Drawing Corrections**

Page 4

Applicant(s): Watowich et al.

Serial No. 09/981,286

Confirmation No. 4993

Filed: October 15, 2001

For: DRUG DISCOVERY METHODS

Remarks

The specification is amended to identify two nucleic acid sequences with SEQ ID NOs. The specification is also amended to correct a typographical error and is supported by the specification at, for instance, page 11, lines 18-28.

The drawings are amended to identify SEQ ID NOs., and to correct inadvertently omitted text.

Conclusion

The Examiner is invited to contact Applicants' Representatives at the below-listed telephone number, if there are any questions regarding this Preliminary Amendment or if prosecution of this application may be assisted thereby.

CERTIFICATE UNDER 37 C.F.R. 1.8:

The undersigned hereby certifies that this paper is being deposited in the United States Postal Service, as first class mail, in an envelope addressed to: Assistant Commissioner for Patents, Attn: Missing Parts, Washington, D.C. 20231, on this 28th day of January, 2002.

David L. Provence
David L. Provence

Respectfully submitted,

Watowich et al.

By
Mueiting, Raasch & Gebhardt, P.A.
P.O. Box 581415
Minneapolis, MN 55458-1415
Telephone: (612) 305-1220
Facsimile: (612) 305-1228

January 28, 2002

Date

By: David L. Provence
David L. Provence
Reg. No. 43,022
Direct Dial (612) 305-1005

**APPENDIX A - SPECIFICATION/CLAIM AMENDMENTS
INCLUDING NOTATIONS TO INDICATE CHANGES MADE**

Serial No.: 09/981,286
Docket No. 265.00260101

Amendments to the following are indicated by underlining what has been added and bracketing what has been deleted. Additionally, all amendments have been shaded.

In the Specification

The paragraph beginning at page 20, line 20, has been amended as follows:

The tat-CCD construct was produced by PCR using CCD in the pET30 vector and the primers N-TATCCD

(5' ATGTACGGTCGTAAAAACGTCGTCAGCGTCGTCGTGTCATGAAATTGGAATCTG ACA3' **SEQ ID NO:35**) and CBAM-VEE

(5' GAATTCGGATCCTCATTACCATGCTCGCAGTTCTCCGGAGT3' **SEQ ID NO:36**).

The PCR product was phenol-chloroform extracted and was ligated into the pETBLUE vector. It was then transformed into NovaBlue Singles (Novagen) and plated on LB-Bluogal-IPTG-carbenicillin-tetracycline plates. White colonies were selected for amplification, plasmid purification, and sequencing. The tat-CCD cDNA sequence was determined and is depicted in Figure 3.

The paragraph beginning at page 26, line 28, has been amended as follows:

Additional approaches to constructing the insert containing the library are also being used. One of these approaches involves annealing a negative strand of LIB (termed LIB r/c) to LIB itself. The LIB r/c sequence was (5' TCGAGGGAACCACC(MNN)mACCACCGGAG (SEQ ID NO:25)), where M= C, A. When LIB and LIB r/c were annealed, cohesive ends for BamHI and XhoI are formed. Another approach is to use Sequenase V 2.0 (USB, Cleveland, Ohio) to synthesize the negative LIB strand. The oligos for this are LIBSEQBAM (5' GCACGGATCCTCCGGTGGT(NNK)**m[o]**GGTGGTTCCTCGAGATCG (SEQ ID NO:26)) and SEQBAM Rev (5' CGATCTCGAGGGAACCATC (SEQ ID NO:27)). This sequenase product is then digested with BamHI (Promega, Madison, WI), and XhoI for insertion into the tat-CCD:BAM expression vectors.



04981236 .021502

Alignment of adaptein nucleotide sequences with CCD sequence:

SEQ ID NO: 31A-1 GTCATGAAAT TGGAAATCTGA CAAGACGTTT CCAATCATGT TGGAAAGGGAA
 SEQ ID NO: 32A-2 GTCATGAAAT TGGAAATCTGA CAAGACGTTT CCAATCATGT TGGAAAGGGAA
 SEQ ID NO: 12 CCD GTCATGAAAT TGGAAATCTGA CAAGACGTTT CCAATCATGT TGGAAAGGGAA

A-1 GATAAACGGC TACGCTTGTG TGGTCGGAGG GAAGTTATTC AGGCCGATGC
 A-2 GATAAACGGC TACGCTTGTG TGGTCGGAGG GAAGTTATTC AGGCCGATGC
 CCD GATAAACGGC TACGCTTGTG TGGTCGGAGG GAAGTTATTC AGGCCGATGC

A-1 ATGTGGAAGG CAAGATCGAC AACGACGTTT TGGCCGCGCT TAAGACGAAG
 A-2 ATGTGGAAGG CAAGATCGAC AACGACGTTT TGGCCGCGCT TAAGACGAAG
 CCD ATGTGGAAGG CAAGATCGAC AACGACGTTT TGGCCGCGCT TAAGACGAAG

A-1 AAAGCATCCA AATACGATCT TGAGTATGCA GATGTGCCAC AGAACATGCG
 A-2 AAAGCATCCA AATACGATCT TGAGTATGCA GATGTGCCAC AGAACATGCG
 CCD AAAGCATCCA AATACGATCT TGAGTATGCA GATGTGCCAC AGAACATGCG

A-1 GGCCGATACA TTCAAATACA CCCATGAGAA ACCCCAAGGC TATTACAGCT
 A-2 GGCCGATACA TTCAAATACA CCCATGAGAA ACCCCAAGGC TATTACAGCT
 CCD GGCCGATACA TTCAAATACA CCCATGAGAA ACCCCAAGGC TATTACAGCT

A-1 GGCATCATGG AGCAGTCCAA TATGAAAATG GGC GTTTT CAC GGTGCCGAAA
 A-2 GGCATCATGG AGCAGTCCAA TATGAAAATG GGC GTTTT CAC GGTGCCGAAA
 CCD GGCATCATGG AGCAGTCCAA TATGAAAATG GGC GTTTT CAC GGTGCCGAAA

A-1 GGAGTTGGGG CCAAGGGAGA CAGCGGACGA CCCATTCTGG ATAACCAGGG
 A-2 GGAGTTGGGG CCAAGGGAGA CAGCGGACGA CCCATTCTGG ATAACCAGGG
 CCD GGAGTTGGGG CCAAGGGAGA CAGCGGACGA CCCATTCTGG ATAACCAGGG

A-1 AGGGGTGGTC GCTATTGTGC TGGGAGGTGT GAATGAAGGA TCTAGGACAG
 A-2 AGGGGTGGTC GCTATTGTGC TGGGAGGTGT GAATGAAGGA TCTAGGACAG
 CCD AGGGGTGGTC GCTATTGTGC TGGGAGGTGT GAATGAAGGA TCTAGGACAG

(HindIII) (XhoI)
 A-1 CCCTTTCAGT CGTCATGTGG AAC---AAGCTT TCTCCACATTA TGCTCAA CTCGA G
 A-2 CCCTTTCAGT CGTCATGTGG AAC---AAGCTT AGAAGCGGTAC TCAATGG CTCGA G
 CCD CCCTTTCAGT CGTCATGTGG AACGAG-----

A-1 ---GGAGTTA CCGTGAAGTA TACTCCGGAG AACTGCGAGC AATGGTAATGAGC
 A-2 ---GGAGTTA CCGTGAAGTA TACTCCGGAG AACTGCGAGC AATGGTAATGAGC
 CCD AAGGGAGTTA CCGTGAAGTA TACTCCGGAG AACTGCGAGC AATGGTAATGAGC

Figure 2B

Figure 2C

Alignment of adaptein protein sequences with CCD sequence:

SEQ ID NO: 33	A-1	VMKLESDKTF	PIMLEGKING	YACVVGGKLF	RPMHVEGKID	NDVLAALKTK
SEQ ID NO: 34	A-2	VMKLESDKTF	PIMLEGKING	YACVVGGKLF	RPMHVEGKID	NDVLAALKTK
SEQ ID NO: 1	CCD	VMKLESDKTF	PIMLEGKING	YACVVGGKLF	RPMHVEGKID	NDVLAALKTK
	A-1	KASKYDLEYA	DVPQNMRA DT	FKYTHEKPQG	YYSWHHGAVQ	YENGRFTVPK
	A-2	KASKYDLEYA	DVPQNMRA DT	FKYTHEKPQG	YYSWHHGAVQ	YENGRFTVPK
	CCD	KASKYDLEYA	DVPQNMRA DT	FKYTHEKPQG	YYSWHHGAVQ	YENGRFTVPK
	A-1	GVGAKGDSGR	PILDNQGRV V	AIVLGGVNEG	SRTALSVVMW	N-KLSPHYA QLE
	A-2	GVGAKGDSGR	PILDNQGRV V	AIVLGGVNEG	SRTALSVVMW	N-KLRSGTQWLE
	CCD	GVGAKGDSGR	PILDNQGRV V	AIVLGGVNEG	SRTALSVVMW	NE-----
	A-1	-GVTVKYTPE	NCEQW			
	A-2	-GVTVKYTPE	NCEQW			
	CCD	KGVTVKYTPE	NCEQW			